What do we (need to) know about the kinetic properties of nanoparticles in the body?

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Abstract

Nowadays the development and applications of nanotechnology are of major importance in both industrial and consumer areas. However, the knowledge on human exposure and possible toxicity of nanotechnology products is limited. To understand the mechanism of toxicity, thorough knowledge of the toxicokinetic properties of nanoparticles is warranted. There is a need for information on the absorption, distribution, metabolism and excretion (ADME) of nanoparticles and validated detection methods of these man-made nanoparticles. Determination of the ADME properties of nanoparticles requires specialised detection methods in different biological matrices (e.g. blood and organs). In this paper, the current knowledge on the kinetic properties of nanoparticles is reviewed. Moreover, knowledge gaps from a kinetic point of view (detection, dose, ADME processes) are identified.
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1. Introduction

The prefix nano is derived from the Greek word nanos, meaning dwarf. Today, nano denotes one-billionth (10^{-9}) of a metre (SI length unit). Nanotechnology represents the design, production and application of materials at atomic, molecular and macromolecular scales, in order to produce new nanosized materials. Arbitrarily, nanomaterials are defined as materials containing man-made nanoparticles or having nanostructured surface topography. Nanoparticles have at least one dimension in the order of 100 nm or less (The Royal Society and The Royal Academy of Engineering, 2004). Nanotechnology has emerged rapidly during the past years in a broad range of product domains. It provides opportunities for the development of materials, including those for medical applications, where conventional techniques may reach their limits. Industry and governments worldwide have invested heavily in nanoscience and further investments are planned in the near future (Service, 2005; Nel et al., 2006). Nanotechnology will have an impact on numerous aspects of modern society. Indeed, consumer products containing nanoparticles are commercially available already (Maynard and Michelson, 2005). The accumulating investments and efforts in this promising technology are expected to lead to new technological breakthroughs and might lead to an increase in the application of these innovative nanomaterials in medical and consumer products in the near future (Roszek et al., 2005).

Nanoparticles show remarkable structural diversity, each structure exhibiting their own individual characteristics, such as tubes, dots, wires, fibers and capsules (Singh et al., 2006; Ballou et al., 2004; Roszek et al., 2005). All

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these nanoscale materials have physical, chemical, optical, electrical, catalytical and mechanical properties that may differ fundamentally from their corresponding macrosized counterparts (Preining, 1998). Consequently, it is reasonable to assume that these deviations in properties can also lead to a different behaviour in the body. In addition, not only the dose expressed as mass, but also particle size, particle numbers, surface area and other characteristics (like charge, shape, etc.) determine the biological interactions of these nanoparticles. Indeed, several studies suggest that these nanoparticles have different toxicity profile compared with larger particles (Oberdorster et al., 2005b; Donaldson et al., 2001). For particles with decreasing sizes, the ratio between the relative surface area and its volume (or mass) will increase rapidly. Since this surface area can interact with biological components of cells, nanoparticles can be more reactive in comparison with larger particles (Brown et al., 2001; Oberdorster, 2001), which increases the potential human health risk of nanoparticles. In addition, the distribution of nanoparticles in the body seems to be size-dependent (de Jong et al., submitted for publication; Hillyer and Albrecht, 2001). Hence, the adverse effects of nanoparticles may not be predicted or derived from known toxicity of the chemical constituent or macroscopic sized counterparts (Oberdorster et al., 1994; Oberdorster et al., 2005b; Brown et al., 2001; de Jong et al., 2005). As a consequence, it is difficult to extrapolate present human risk assessment data from macrosized chemicals to risk assessment for the corresponding nanoparticles of the same chemical composition. However, at this time, no specific safety guidelines for the production and use of nanoparticles and nanotechnology are available. To date, in general the normal regulations for chemical substances apply to nanotechnology products. Currently, there is an exception, which deals with silver nanoproducts sold as germ killing agents in the USA. For biocidal products specific regulations exist, for which the producers have to prove that the biocide, in this case nanosized silver, has no adverse effects on humans and the environment (The Washington Post, 2006).

In order to assess the potential risks for human health the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) identified some major knowledge gaps that need to be addressed to improve risk assessment for products produced through nanotechnology (Scientific Committee on Emerging and Newly Identified Health Risk (SCENIHR), 2005). One of their recommendations is that the mechanisms of release and kinetics of nanoparticles from a very wide range of production processes, formulations and usage of the nanotechnology products need to be characterized. In addition, the actual range of exposure levels to nanoparticles needs to be assessed and the extent to which it is possible to use or extrapolate data from the toxicology of larger particles of the same chemical substance. Another important knowledge gap lies in the fate, distribution, persistence and bioaccumulation of nanoparticles in humans. Target organs

and toxic responses should be identified and realistic exposure levels for risk assessment should be determined following exposure to nanoparticles. This includes dose response data for the target organs, and knowledge of the subcellular location of nanoparticles and the mechanism of their interaction at the cellular level (Scientific Committee on Emerging and Newly Identified Health Risk (SCENIHR), 2005).

However, to date, almost all toxicological experiments dealing with nanoparticles describe the external exposure. External exposure describes the total ingested, inhaled or dermally applied dose of nanoparticles. The internal exposure is the part of the external dose of nanoparticles that reaches the systemic circulation and other organs and tissues (Fig. 1). From a toxicological perspective, a toxic effect is only provoked if sufficient quantities of (an active metabolite of) substances or particles reach a target site (receptor, cell or organ), which in turn is a function of the duration of exposure and dose rate.

To quantify the probability that harmful effects of nanoparticles occur in human health and the environment, proper risk assessment is pivotal. Identification of the effects caused by the particles as well as a dose–response relationships are needed for risk assessment. Sufficient information on absorption, distribution, metabolism and excretion (ADME) of the nanoparticles may be needed for various extrapolations (cross dose, cross species and route-to-route). For ordinary chemical substances, toxicokinetic and toxicodynamic information needed for risk assessment is already present or might be predicted based on structural analogues. For nanoparticles, however, this information is scarce or even absent. Due to this lack of information, appropriate risk assessment is accompanied by large uncertainties. Besides toxicodynamic studies for nanoparticles, additional toxicokinetic research is required for several types of nanoparticle "case by case" to ensure their safe application, until it is possible to make more general assumptions.

The scope of this review is to survey existing knowledge regarding the kinetic properties of nanoparticles and to identify and address the most urgent gaps in the understanding of the kinetics of these nanoparticles. The toxicological property of nanoparticles was not our primary focus. There are already excellent reviews that discuss the current knowledge of nanoparticle toxicity (Oberdorster et al., 2005a,b; Borm et al., 2006; Kreyling et al., 2006). This review will only refer to the toxic properties of nanoparticles when it will contribute substantially to explain the kinetic processes of nanoparticles.

2. Detection

Table 1 shows a variety of measurements describing the physicochemical properties of nanoparticles used in several studies. Remarkably, almost all studies describe the properties of the analysed nanoparticles in a different way. To date, there is no agreed standardised approach for the
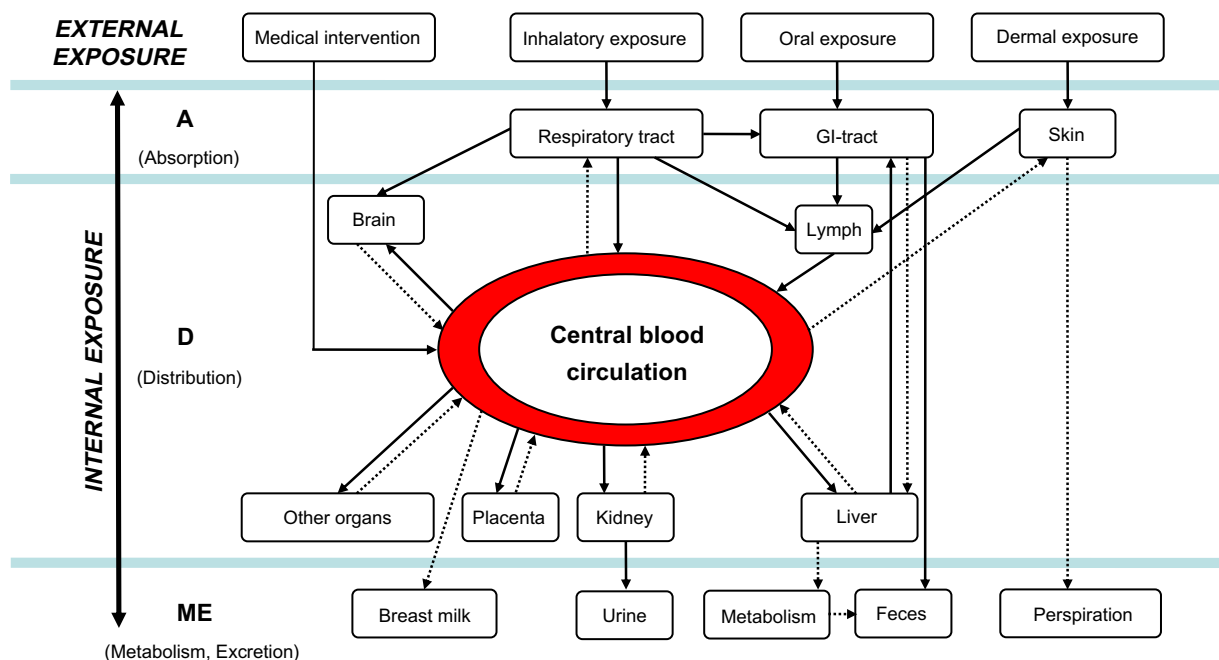
Fig. 1. Kinetic properties of nanoparticles in the body. In this scheme, the ADME processes (absorption, distribution, metabolism and excretion) are indicated. The internal exposure is the part of the external dose that reaches the systemic circulation. The black lines represent confirmed routes for nanoparticles; the dashed lines represent hypothetical routes. The transport rates and retention times for the indicated processes are largely unknown (other organs: e.g. spleen, heart, reproductive organs. Modified from Oberdorster et al. (2005b) Environmental Health Perspective).

measurement of nanoparticles (Oberdorster et al., 2005a; Tsuji et al., 2006). Since proper analytical detection is a crucial prerequisite to determine the kinetics of nanoparticles, new analytical disciplines have emerged. These methods will help to answer the two fundamental questions.

1. How is the dose of a nanoparticle defined?
2. Is it possible to measure the internal dose?

2.1. How is the dose of a nanoparticle defined?

To date, studies with nanoparticles describe the best dose–response correlation by measuring at least the three principal physical parameters, which are mass, surface area and number concentration of nanoparticles (Tabel 1; Oberdorster et al., 2005a). In a recent study, the number of particles, the product of particle number and mean size and the surface were explored to quantify the lung inflammatory response to nanoparticle exposure. As a result, all three dose metrics worked quite well to describe the nanoparticle dose (Wittmaack, 2007). Since the contribution of charge (Veronesi et al., 2002) and other physicochemical properties (chemical composition, structure, solubility) could also influence the dose–response relationship of nanoparticles to some extent (Oberdorster et al., 2005a,b), relevant particle properties should also be analyzed and reported.

However, the description of a nanoparticle dose sometimes depends on simplifications and assumptions. For example, it is sometimes unknown if the particles size

distribution is monodisperse (all particles are of the same geometric size) or polydisperse (the distribution contains a range of sizes). If the size distribution is polydispersed with the size expressed as median size, an integral of the particle size distribution will increase the accuracy of the description of the dose. In addition, the total "effective" surface area of nanoparticles decreases due to aggregation and agglomeration of particles. Therefore, it would be useful to asses the degree of aggregation and agglomeration. Moreover, it would even be more relevant to address the "effective" surface area of nanoparticles (inside biological matrixes).

These uncertainties, together with the distinct physico-chemical properties of different nanoparticles makes it a scientific challenge to describe the dose in such a way that doses of various types of nanoparticles (e.g. spheres, rods, dendrimers, etc.) can be compared in a quantitative manner.

2.2. Is it possible to measure the internal dose?

To date, nanoparticles can be analysed by various methods. These methods include electron microscopy, spectroscopy, diffraction analysis and a surface analysis (Table 2). However, there is no single detector capable of detecting all of the important particle characteristics. As a result, nanoparticle characterization requires a fully equipped and specialized lab. Samples taken at places of interest should be analyzed before storage, decreasing the effects of storage conditions on the particle characteristics.

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Table 1
The variety of measurements describing the physicochemical properties of nanoparticles used in several studies

Nanoparticle	Dose	Particle number	Surface area	Size	Geometrical SD	Aggregates/ agglomerates	Charge	Additional detection	Route	Animal	Reference
Ultrafine carbon particles	+	+		+	+				Inhalation	Mouse	Andre et al. (2006)
Technetium (Tc)-labelled Carbon	+			+		Reported		Isotope	Inhalation	Human	Brown et al. (2002)
C$_{82}$ fullerene								Isotope	Intravenous	rat	Cagle et al. (1999)
PLGA particles				+	+			EM	Gastrointestinal uptake	Rat	Desai et al. (1996)
High and Low surface Carbon black	+		+	+					Inhalation	Hamster, rat, mouse	Elder et al. (2005)
Magnese oxide particles				+		Reported		EM	Intranasally instillation	Rat	Elder et al. (2006)
ZnO; TiO$_2$ particles	+			+	+	Reported		EM	Dermal	Pig skin	Gamer et al. (2006)
Colloidal gold particles				+	+				Oral uptake	Mouse	Hillyer and Albrecht (2001)
Fluorescent particles				+					Dermal	Pig skin	Kohli and Alpar (2004)
Ultrafine 192 Iridium particles	+	+		+	+	Reported		EM, Isotope	Inhalation	Rat	Kreyling et al. (2002)
Silver particles				+				EM	In vitro	Antibacterial effect	Lee et al. (2003)
QD Cadmium Telluride (CdTe)				+	+		+		In vitro	in vitro	Lovric et al. (2005)
Tc-labelled Carbon particles				+	+	Reported		EM	Inhalation	Human	Mills et al.(2006)
Tc albumin nanocolloid particles	+	+		+				Isotope	Intratracheal instillation	Hamster	Nemmar et al.(2001)
Tc-labelled Carbon particles				+		Reported		Isotope	Inhalation	Human	Nemmar et al. (2002)
Polystyrene particles	+			+					Intratracheal instillation	Hamster	Nemmar et al. (2003)
Polystyrene particles	+			+		Reported	+	EM	Intra-tracheal and venous	Hamster	Nemmar et al. (2003)
Ultrafine 13 Carbon	+			+	+			Isotope	Inhalation	Rat	Oberdorster et al. (2004)
Fluorescein-labelled polystyrene	+			+					Intravenous	Rat	Ogawara et al. (1999a)
SiO$_2$; TiO$_2$; Co; Ni; PVC particles	+			+	+			EM	In vitro	In vitro	Peters et al. (2004)
C$_{60}$ fullerene								Isotope	Intravenous	Mouse	Qingnuan et al. (2002)
C$_{60}$ fullerene	+							HPLC	Intravenous	Rat	Rajagopalan et al. (1996)
C$_{60}$ fullerene						Reported			In vitro	In vitro	Sayes et al. (2004)
TiO2 particles	+			+				EM	Dermal	Human skin biopt	Schulz et al. (2002)
Ultrafine 192 Iridium particles		+		+				Isotope	Inhalation	Rat	Semmler et al. (2004)
Single/multiwalled carbon nanotubes				+				EM, isotope	Intravenous	Rat	Singh et al. (2006)
C$_{60}$ fullerene	+								Intraperitoneal	Mouse	Tsuchiya et al.(1996)
Tc-labelled Carbon particles		+		+	+			Isotope	Inhalation	Human	Wiebert et al.(2006)

Note that almost all studies describe the properties of the used nanoparticles in a different way.

Another drawback of the majority of the analysis methods is that the detection should be performed in a pure solution, without impurities originating from biological samples. However, these samples contain a wide variety of biological impurities and contaminations (e.g. proteins, salt) in the sample and this might interfere with quantitative detection of nanoparticles inside this mixture. An overview of detection methods commonly used to characterize nanoparticles are listed in Table 2 (Powers et al., 2006).

For better insight into the relationship between the nanoparticle dose and the induced effect on various organs in the body, it is important to measure the nanoparticles inside the body (Fig. 1). This means that new characterization techniques are needed that enable quantitative measurement of nanoparticles in these biological matrices (e.g. saliva, blood, urine, faeces and inside organs). Recently, several analytical techniques have become available which allow quantitative detection of for instance fullerenes (Xia et al., 2006) and gold nanoparticles (Hillyer and Albrecht, 2001) in biological media. These methods first extract the nanoparticle from the biological sample, after which the concentration of the extracted nanoparticle is quantitatively determined by high-performance liquid chromatography or mass spectrometry. These methods will only give a reliable determination if the extraction procedure for nanoparticles is validated.

Other methods to detect nanoparticles in urine, blood and inside other organs by electron microscopy analysis have been published recently. However, these methods are not quantitative and can therefore only provide information concerning the presence or absence of nanoparticles in the biological matrix (Gatti et al., 2004; Gatti, 2004; Singh et al., 2006).

The reproducibility and validation of these methods for the detection of nanoparticles is of utmost importance. The nanoparticles detected should be in the linear range of the analysis method. Therefore the lower limit of detection,

lower limit of quantification and the higher limit of quantification must be appropriate for the analysis used to quantify the nanoparticles. Also day-to-day, within day, batch-to-batch and interlaboratory variations of the analysis method as well as the storage conditions of the particles should be taken into account. Effective implementation of these considerations would enhance the quality of results significantly and therefore lead to reliable conclusions. However, the variety of different nanoparticles and different biological matrices makes quantitative detection difficult since each different combination requires unique measurement techniques. Until validated quantitative characterization methods become available, mass-balance, radioactive label or isotopic tracing studies (Gulson and Wong, 2006) might give some insight in kinetic parameters like clearance, half-life and distribution of the particles in representative models.

3. Relevant routes of exposure

The potential applications of nanoparticles in food products, drug-delivery systems, medical devices, consumer products and the increasing disposal of nanoparticles in the environment imply that human exposure to nanoparticles is expected to increase in the (near) future.

The release of nanoparticles in the environment as aerosols originating from traffic, waste and (nanotechnology) industry processes (e.g. workers exposure) suggests that inhalation represents an important route for human exposure to nanoparticles. Another source of exposure for the population may be the (future) waste disposal of nanotechnology derived products. This disposal could eventually lead to increased particle concentration in soil and (drinking) water sources. In addition, application of nanoparticles in products such as medical products, cosmetics and food will result in exposure of the skin and gastrointestinal tract. For the various nanotechnological applications, different exposure routes including the inhalation, oral, dermal, parenteral route and implantation will need attention in the near future.

In Fig. 1, the potential lifecycle of nanoparticles in the human body is represented schematically. From a kinetic point of view, this figure gives an overview of the ADME processes in the body. Fig. 1 also indicates that particles can be distributed to the same organ by several routes of exposure. For example, nanoparticles found in the gastrointestinal tract can originate from ingested products. On the other hand, they can also reach the gastrointestinal tract via an indirect route. For example, via the respiratory tract or the skin, particles can be absorbed in the systemic circulation. From there, particles can be distributed to the liver, taken up by hepatocytes and excreted in the bile to the gastrointestinal tract. This example shows clearly that it is necessary to conduct kinetic experiments if the exact route (exposure, absorption, distribution, metabolism and excretion) of a particle in the body has to be elucidated in detail.

Table 2
Detection methods, currently used for the detection and characterization of nanoparticles

Particle characteristic	Possible analysis method	Possible in biological sample?
Particle number	Condensation particle counting	No
Surface area	Differential mobility analysis/diffusion charge	No
Particle size	Dynamic light scattering	Yes
Particle mass	Particle mass analysis	No
Particles structure	Electron/X-ray diffraction	Yes (after washing)
Particle surface charge	Zseta Potential	No
Visualization (morphology)	Electron microscopy (SEM, TEM)	Yes (after fixation of tissue)
Location (inside the body)	Radioactive labeling of nanoparticles	Yes

More detection methods are reviewed by Powers et al. (2006).

4. Absorption

In this section, absorption of nanoparticles into the human body via different exposure pathways (inhalation, oral, skin and parenteral) is addressed. In pharmacokinetics, absorption represents the process by which unchanged compounds (e.g. nanoparticles) proceed from the site of administration to the central blood circulation (site of measurement).

4.1. Absorption via the lung

In inhalation toxicology, the term translocation is often used to describe particle uptake from the lung and deposition of the particle in target organs. However, from a kinetic point of view, translocation represents both the absorption and distribution process. In this review, these processes are addresses separately.

The respiratory system represents the main port of entrance for airborne nanoparticles. Particle deposition in the respiratory tract depends on various factors including particle size, breathing force and the structure of the lungs. In addition, due to the small diameter of the nanoparticles, Brownian diffusion also determines deposition, resulting in a deep penetration of nanoparticles in the lungs and diffusion to the high lung surface area presented in the alveolar region. These deposition processes results in minimal deposition probability curves (Fig. 2) in the lungs of particles with a diameter of between 100 nm and 1 μm (Maynard and Kuempel, 2005). Deposition can be modelled based on size (ICRP (International Commission on Radiological Protection), 1994; Price et al., 2002) by means of mathematical models (Multiple-Path Particle Dosimetry Model (Price et al., 2002)) which indicate that more than 80% of the nanoparticles inhaled (<100 nm) may be deposited in the respiratory tract (ICRP (International Commission on Radiological Protection), 1994; Fig. 2). Nanoparticles in the low nanometer region are expected to be deposited also in the upper airways due to strong diffusion before transportation into the deep lung (Maynard and Kuempel, 2005).
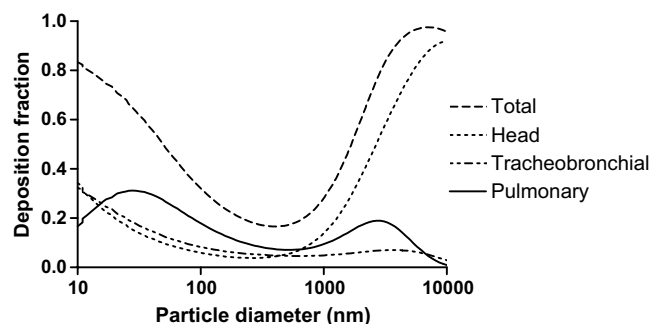

Fig. 2. The modelled deposition of particles (10 nm–10 μm) in the respiratory tract. The deposition is modelled with the Multiple-Path Particle Dosimetry Model (MPPD) assuming an adult nasal breathing at 7.5 l/min. Below 10 nm, reliable prediction is not possible with this model. Calculated with MPPD v 1.0 (2002) RIVM report 650010030.

Several epidemiological studies have focussed on particles in the nanosize range and its negative effects on human health (Vermylen et al., 2005; Peters et al., 2006). Interestingly, the ultrafine particles in polluted air, when inhaled, appear not only to increase respiratory diseases as a local effect, but also have a systemic effect, resulting in a significant increase in cardiovascular risk in morbidity and mortality (Vermylen et al., 2005). However, it is not clear if the systemic effects are always a result of nanoparticles themselves or the cascade of events that are initiated by nanoparticles in the lung. The potential of nanoparticles for inducing systemic effects in the cardiovascular system was supported by results of Nemmar et al. (2002), demonstrating a rapid absorption of inhaled ^{99m}Technetium-labelled carbon nanoparticles in the lungs of healthy humans (Nemmar et al., 2002). However, these findings could not be reproduced in comparable studies using the same label (Brown et al., 2002; Wiebert et al., 2006; Mills et al., 2006). It was argued that the studies of Nemmar et al. (2002) reported the absorption of ^{99m}Tc species rather than the ^{99m}Tc-radiolabelled nanoparticles.

In addition, animal studies have demonstrated low but detectable levels of absorption of nanoparticles from the lungs (Geiser et al., 2005; Kreyling et al., 2002; Oberdorster et al., 2002). In animal inhalation studies, the limit of detection is lower compared to human inhalation studies since the target organs in animals can be examined *ex-vivo* (Oberdorster and Elder, 2007).

4.2. Absorption via the olfactory nervous system

There is evidence for an additional absorption route for inhaled nanoparticles. Oberdorster et al. (2004) reported significant absorption of carbon nanoparticles from the olfactory mucosa of the respiratory tract into the central nerve system via the olfactory nerve in rats (Oberdorster et al., 2004). This absorption route has already been described in 1940 for nanosized polio virus particles (Howe and Bodian, 1940). A recent study demonstrated that inhaled manganese oxide particles were absorbed via the olfactory neural pathway in rats (Elder et al., 2006). These studies suggest that this pathway represents an additional port of entry for nanoparticles into the brain, which circumvents the restricted blood–brain barrier (Oberdorster et al., 2005b; Oberdorster et al., 2004). Neuronal absorption may vary depending on the nature (chemical composition, size, charge) of the nanoparticles and may vary from species to species (rats and humans; Oberdorster et al., 2005b). Although the neuronal absorption route has not yet been confirmed in humans, this route is likely to be operative in humans (Elder et al., 2006). Therefore, more research is required to determine the significance of absorption of nanoparticles to the central nerve system and the possible effects on the brain (Peters et al., 2006).

The absorption of nanoparticles via the olfactory nerve is not only of interest from a toxicological point

of view (Borm and Kreyling, 2004). This nanoparticle absorption route is also studied for the development of drug-delivery systems. These systems might circumvent the blood–brain barrier in order to enable distribution of diagnostics or medical therapeutics to the brain (Olivier, 2005).

4.3. Gastrointestinal absorption

The gastrointestinal tract may represent an important port of entry for nanoparticles since food products may eventually contain nanoparticles (Lomer et al., 2002; Maynard and Michelson, 2005). Also inhaled particles can be excreted via the mucociliary escalator and subsequently be ingested into the GI tract. The contribution of oral exposure following inhalation exposure depends on the physicochemical characteristics and size of particles. The computer model Multiple-Path Particle Dosimetry Model (Price et al., 2002) can predict the amount of poorly soluble non-toxic solid nanoparticles (10 nm and larger) transported from the lungs to the GI-tract in rats. Validated extrapolation to the human situation for nanoparticles is not possible yet.

Microsized particles can enter the body by a process called persorption, the paracellular uptake of particles from the digestive tract into lymphatic and blood circulation (Volkheimer et al., 1968; Volkheimer, 1974). Several studies in rats have shown that nano and microsized particles (50 nm–20 μm) are absorbed mainly through Peyer's Patches of the small intestine, although the Peyer's Patches only comprise a small percentage of the total surface of the small intestine. Absorption via intestinal enterocytes has been demonstrated also (Florence, 2005; Hillery et al., 1994; Jani et al., 1990; Carr et al., 1996). Charge appears to be an important determinant of the extent of absorption. Positively charged particles seem to be absorbed more effectively through the gastrointestinal tract than neutral and negatively charged particles (Janes et al., 2001; Hussain et al., 2001; Florence, 1997; Florence, 2005). In addition, the size of the particles determines the extent of absorption. Polystyrene nanoparticles of 50 and 100 nm were found to be absorbed for 34% and 26%, respectively (Jani et al., 1990). The efficiency of absorption of 100 nm polystyrene particles (4 mg/ml) was found to be up to 250-fold higher compared to larger sized (500 nm, 1 and 10 μm) polystyrene microparticles (4 mg/ml; Desai et al., 1996). However, no data are available that link gastrointestinal absorption of polystyrene nanoparticles to negative effects in rats. In addition, the variety of nanoparticles tested with different physicochemical properties, the heterogeneity in experimental protocols and the higher amount of M-cells in the Peyer's Patches of rodents compared to humans shows the need for proper transport studies in human material (des Rieux et al., 2006). Unfortunately, clinical transport studies with nanoparticles are currently still missing.

4.4. Dermal absorption

Dermal exposure might represent an important nanoparticle absorption route. Cosmetics containing nanoparticles such as sunscreens, are directly applied on the skin. Another exposure source may be textiles or wound dressings which can contain silver nanoparticles for antibacterial effect (Lee et al., 2003; Roszek et al., 2005).

As the skin is easily accessible, the transdermal absorption is well studied in recent vaccine and drug-delivery research projects (Partidos, 2003; Prausnitz et al., 2004). These targeted studies involve delivery of nanoparticles to the dermis by penetration of the epidermis.

Exposure to UV radiation may lead to (severe) cutaneous damage. To protect the skin, nanosized metallic oxides such as titanium dioxide (TiO_2) and zinc oxide (ZnO) are added to sunscreens. These nanosized metallic oxides do not scatter visible light, but rather absorb UV light. This results in a translucent sunscreen that will protect the skin via its UV-absorbing properties.

Although a pilot study of Tan et al. (1996) showed an increase of the level of titanium in the epidermis and dermis after sunscreen use (Tan et al., 1996), other studies have shown that microsized and nanosized TiO_2 and ZnO were unable to penetrate the epidermis (Schulz et al., 2002; Gamer et al., 2006). These experiments did not take the movement of skin, the charge of the particle, follicular openings and gender and age related differences in skin characteristics into account.

Tinkle et al. (2003) showed that penetration of microsized particles (500–1000 nm) through the epidermis occurred after application of a flexing motion (Tinkle et al., 2003). Also a study by Rouse et al. (2007) showed penetration of C_{60} fullerene amino acid through the skin after mechanical flexing (Rouse et al., 2007). This suggests that absorption of nanoparticles at skin creases during movement could be greater than from flat skin.

Kohli and Alpar (2004) have reported the penetration of negatively charged latex particles (50 and 500 nm), while positively charged and neutral particles were not able to penetrate the epidermis at all (Kohli and Alpar, 2004). They concluded that also the charge of nanoparticles is one of the important factors in the transdermal absorption process (Kohli and Alpar, 2004). In addition, quantum dots (spherical: 4.6 nm and ellipsoid: 12 nm by 6 nm) showed penetration through the intact skin (dermis) (Ryman-Rasmussen et al., 2006). This suggests that the skin is permeable to nanomaterials with distinct physicochemical properties (size, shape, charge, material).

The studies reported to date indicate that nanoparticle migration through the skin is possible, especially when mechanical flexion is applied to the skin. The migration of nanoparticles through the dermis suggests that systemic circulation can be reached. However, quantitative data confirming this absorption process is lacking. Almost all experiments are performed on healthy human or porcine skin. However, for sunscreen application, attention should

be paid to the dermal absorption of nanoparticles through damaged, sun burned, dried skin, skin in motion and children's skin (de Zwart et al., 2004; West et al., 1981).

5. Distribution

In this section, the distribution of nanoparticles via central blood circulation to the various tissues and organs is addressed. After the absorption process of nanoparticles by the various ports of entry, the systemic circulation can distribute them towards all organs and tissues in the body. Several studies have shown distribution of particles to several organs including liver, spleen, heart and brain (Ji et al., 2006; Nemmar et al., 2002; Hillyer and Albrecht, 2001; Oberdorster et al., 2002).

5.1. Systemic circulation

When nanoparticles reach the systemic circulation, the particles can, potentially, interact with plasma–proteins, coagulation factors, platelets and red and white blood cells. Especially, the binding to plasma components may have a substantial effect on the distribution and excretion of nanoparticles. The addition of serum *in vitro* resulted in a reduction in silica particle induced cytotoxic effects. A specific serum component, apolipoprotein-A1 (apo-A1), was identified to bind the silica, but apo-A1 could also bind to other particles (*eg.* asbestos, TiO_2) (Barrett et al., 1999). In addition, quantum dots (QDs) pre-treated with bovine serum albumin showed a reduction in the induction of cell death *in vitro (*Lovric et al., 2005). Also the subcellular localization of albumin modified QDs was different than unmodified QDs (Lovric et al., 2005). An explanation for the reduction in toxicity in the presence of albumin could be the scavenging ability of albumin (Moosmann and Behl, 2002). The presence of albumin and apo-A1 in human serum might inhibit (to some extent) the toxic effects of nanoparticles in the systemic circulation. It is not known whether the interaction between the proteins and nanoparticles is reversible. Furthermore, the fraction of unbound nanoparticles should be determined, since this fraction might correlate better with the toxicity observed. Therefore, more research is required to examine the interaction of proteins with nanoparticles. Caution should be exercised in the extrapolation of *in vitro* studies to *in vivo* situations, since the influence of serum is not taken into account in most of the *in vitro* studies.

Nanoparticles have been identified in the systemic blood circulation (Gatti et al., 2004; Hillyer and Albrecht, 2001). In addition, several different nanoparticles (gold and titanium oxide) have been identified inside human red blood cells (Rothen-Rutishauser et al., 2006). Interestingly, this cellular uptake of nanoparticles did not involve endocytosis (Geiser et al., 2005) since erythrocytes do not have phagocytotic receptors (Rothen-Rutishauser et al., 2006). This suggests that nanoparticles are able to cross the cell membrane by processes other than phagocytosis and endocytosis. Diffusion, transmembrane channels, adhesive interactions or other, undefined, transmembrane processes might play an important role in this cellular uptake. The intracellular gold and titanium oxide nanoparticles are not membrane bound and might have direct access to the intracellular proteins, organelles and DNA of the cell. This process could enhance their toxic potential (Geiser et al., 2005).

5.2. Distribution following inhalation exposure

The previously discussed study of Nemmar et al. (2002) revealed a rapid and significant distribution of inhaled ^{99m}Technetium-labelled carbon nanoparticles from the systemic circulation to the liver of healthy humans (Nemmar et al., 2002). These findings could not be replicated with similar human studies using the same label (Brown et al., 2002; Wiebert et al., 2006; Mills et al., 2006). Interestingly, other studies in animals showed (low, but detectable) distribution of inhaled radiolabelled particles to organs including liver, heart, kidney, spleen and brain, suggesting distribution via blood circulation (Oberdorster et al., 2002).

In conclusion, the distribution of inhaled nanoparticles via the systemic circulation to other organs has been shown (Nemmar et al., 2002, 2001; Oberdorster et al., 2002; Kreyling et al., 2002), although the results of the human studies could not be reproduced by others (Brown et al., 2002; Wiebert et al., 2006; Mills et al., 2006). The lower detection limit in animal studies compared to human studies might explain the different outcomes of the animal and human studies (Oberdorster and Elder, 2007). Therefore, further kinetic and toxicokinetic studies are required to determine the existence and extent of the distribution of nanoparticles after inhalation.

5.3. Distribution following oral exposure

Hillyer and Albrecht showed that after oral administration of metallic colloidal gold nanoparticles of decreasing size (58, 28, 10 and 4 nm) to mice an increased distribution to other organs was observed. The smallest particle (4 nm) administered orally resulted in an increased presence of gold particles in kidney, liver, spleen, lungs and even the brain. The biggest particle (58 nm) tested was detected almost solely inside the gastrointestinal tract (Hillyer and Albrecht, 2001). This suggests that both the absorption (Jani et al., 1990) and distribution (Hillyer and Albrecht, 2001) of nanoparticles are size-dependent.

5.4. Distribution following intravenous dosing and from implants

Modern medical applications could represent another port of entry for nanoparticles in the body. Nanoparticles could be injected directly into the body as contrast agents for imaging purposes or for drug-delivery applications. Also, nanoparticles could inadvertently arise due to wear

from implanted biomaterials (Gatti and Rivasi, 2002). In the near future, an increasing number of applications of nanotechnology in healthcare is foreseen, which indicates that exposure to nanoparticles inside the body will increase (Roszek et al., 2005).

Quantum dots (QDs) are semiconductor nanocrystals (2–100 nm) with unique optical and electrical properties. There are a wide range of different QDs available, consisting of a metalloid core, surrounded by a cap that shields the core. This cap can be functionalized to enhance for instance the QD water solubility, durability or bioactivity (Hardman, 2006). Also, specific labels and moieties can be coupled to this core, making them applicable for imaging and targeting studies (Alivisatos, 2004). Several QDs are applied *in vivo* in biomedical imaging studies already. This implies that QDs are directly injected in the body. The toxicity, however, of these QDs are understood poorly. Lovric et al. (2005) found that size and charge affected the *in vitro* cytotoxicity of QDs (2.2 vs 5.2 nm) in cells significantly (Lovric et al., 2005). However, it is not known whether the coating alone contributes to the toxic effects observed. Also, the toxicity observed *in vitro* did not occur *in vivo* in several experiments (Ballou et al., 2004; Larson et al., 2003). In addition, Ballou found that half-lives of QDs in mice depended on the surface coating (Ballou et al., 2004). QDs that were coated with short peptide chain (750 Da) were distributed within 1 h from the circulation to lymph nodes, liver and bone marrow, whereas long-chain (5000 Da) surface coated QDs remained in the circulation for at least 3 days. These particles were mainly distributed to liver, spleen and bone marrow, while smaller amounts of QDs accumulated in the lymphatic system. Taken together, the kinetics and toxicity of QDs depend on several important factors including size, charge and functional coating (Araujo et al., 1999; Hoshino et al., 2004; Ballou et al., 2004; Lovric et al., 2005). In addition, the elimination process (metabolism and excretion) of QDs are poorly understood. Therefore, further studies are needed to understand the potential risk of these QDs to human health.

Debris resulting from for example wear of hip prostheses can migrate and disseminate to other parts of the body (Revell et al., 1997).These micro- and nanosized wear particles have been found in the blood and in various organs in the body, including liver, kidney and colon (Gatti et al., 2004; Gatti, 2004; Gatti and Rivasi, 2002). These particles, although derived from biocompatible material revealed pro-inflammatory effects on endothelial cells *in vitro* (Peters et al., 2004). This suggests that release of nanosized debris from biocompatible implants might cause inflammatory effects.

5.5. Trans-placental distribution

Data addressing the distribution of nanoparticles to the reproductive cells is, as yet, unavailable. In addition, no clear data is available identifying the distribution of nanoparticles in the foetus. An *in vivo* study in which pregnant mice were intraperitoneally injected with soluble fullerenes C_{60} revealed harmful effects. At high concentrations (137 mg/kg), all the embryos died. Lower concentrations (25–50 mg/kg) resulted in abnormalities especially around the head region (Tsuchiya et al., 1996). This *in vivo* study indicates that C_{60} was transferred into the embryo via trans-placental passage of the maternal blood flow (Tsuchiya et al., 1996). However, direct passage from the peritoneal cavity into the uterus could not be excluded. An *in vitro* study on mouse embryos showed internalization of polystyrene nanoparticles inside the embryo. However, *in vitro* embryo development was not inhibited by these nanoparticles (Bosman et al., 2005). The different outcomes in embryonic responses after exposure to fullerenes (Tsuchiya et al., 1996) and polystyrene nanoparticles (Bosman et al., 2005) show that the chemical composition and concentration (Tsuchiya et al., 1996) of nanoparticles are important for embryo toxicity. These experiments illustrate the need to examine these reproductive areas carefully, since the exposure of nanoparticles to the foetus and reproductive tissue could have dramatic consequences.

5.6. Blood–brain barrier

The blood–brain barrier (BBB) controls the passage of substances from the blood into the central nervous system. The permeability of this physical barrier is highly restricted to molecules which are either lipophillic, actively transported compounds or are small soluble molecules (<500 Da). For nanoparticles, it is not known to what extent they can be distributed across the BBB to the brain from the systemic blood circulation. Evidence exists that this distribution might be relevant, since low concentrations of gold were found in the brain after oral administration of gold nanoparticles (Hillyer and Albrecht, 2001). Further studies are needed to determine the extent of this distribution-route. Indeed, such studies would open the possibility for the development of nanosized drug-delivery systems to the brain (Olivier, 2005).

6. Metabolism

Once nanoparticles are absorbed by the gastrointestinal tract, these particles will be transported directly to the liver via the portal vein. The liver is able to actively remove compounds from the blood. Although for nanoparticles no evidence exists that this "first pass effect" plays a role. In general, this elimination mechanism affects the bioavailability of absorbed compounds in the body.

Studies on metabolism of nanoparticles have not been reported thus far. It is unlikely that inert nanoparticles such as gold and silver particles, fullerenes and carbon nanotubes, can be metabolized effectively by enzymes in the body. However, it could be hypothesised that nanoparticles with functionalized groups can be metabolized. For instance, the protein cap of a functionalized quantum dot could be cleaved by proteases (Hardman, 2006). Also the

metallic core of quantum dots (and other metal oxides) could be bound by metallothionein and excreted. These enzymes, present in liver and kidney, can bind metal and restore the cellular metal homeostasis (Coyle et al., 2002). In addition, nanoparticle drug-delivery systems consisting of liposomes are able to fuse with cell membranes. The intracellularly released drug could be metabolised according to the normal metabolism pathway described for the conventionally formulated drugs.

7. Excretion

Several inhalation studies present clearance data and half-lives of instilled nanoparticles (Elder et al., 2005; ICRP (International Commission on Radiological Protection), 1994; Ferin et al., 1992; Geiser et al., 1990). In these studies, the excretion of nanoparticles represents the particles that are not absorbed into the body, but only cleared from the port of entry, e.g. the lungs. These experiments provide information concerning the clearance mechanism to remove particles from the lung (external exposure) rather then information on systemic clearance of nanoparticles (internal exposure).

An absorbed nanoparticle in the systemic circulation can be excreted by various routes. A possible elimination route for nanoparticles could be renal clearance. Indeed, this route has been found to clear fullerenes and single walled carbon nanotubes (SWCNT) from the body (Singh et al., 2006; Rajagopalan et al., 1996). The plasma half-lives following intravenous injection in rats have been determined and were found to be 6.8 ± 1.1 h for C_{60} fullerenes and 3–3.5 h for functionalized SWCNT (Singh et al., 2006; Rajagopalan et al., 1996). Interestingly, a study with C_{82} fullerenes suggested a prolonged circulation time compared to the C_{60} (Cagle et al., 1999). However, the fullerenes used in these studies have different functionalized groups (Sayes et al., 2004), suggesting that not only size but also the chemical properties of nanoparticles influence its excretion.

In drug targeting, elongation of the circulation time results in a more pronounced accumulation of the drug targeting conjugate at the target site. Coating of nanoparticles with poly ethylene glycol (PEG) gives the nanoparticle a stealth character (Olivier, 2005; Niidome et al., 2006; Bazile et al., 1995).

Intravenously injected quantum dots were still detectable after 133 days in the lymph nodes and bone marrow of mice. This shows that the half-life of QDs might be very long (Hardman, 2006; Ballou et al., 2004). The excretion route for these QDs remains to be elucidated.

An additional excretion route has been suggested for polystyrene nanoparticles. After intravenous administration in rats, these particles were taken up by the liver and subsequently excreted in the bile. The polystyrene nanoparticles (50 nm) were phagocytosed by Kupffercells partly and partly taken up by the hepatocytes (Ogawara et al., 1999b). After 24 h, 4% of the dose was excreted into bile

(Furumoto et al., 2001; Ogawara et al., 1999a). Polystyrene microparticles (500 nm), however, were taken up predominantly by non-parenchymal cells (Kupffercells and endothelial cells) (Ogawara et al., 1999b). This suggests that, depending on the particle size, the polystyrene nanoparticles are partly taken up by the hepatocytes and transported to the bile. It is not known whether metabolism plays a role in this excretion route. Other excretion routes for nanoparticles in the body would be the perspiratory glands and breast milk. However, data are currently not yet available to confirm these routes.

The elimination route of absorbed nanoparticles remains largely unknown and it is possible that not all nanoparticles will be eliminated from the body. Accumulation could take place at several sites in the body. At low concentrations or with single exposure, the toxicity may not be high. However, following a high or chronic exposure, the accumulation of nanoparticles could comprise an additional risk. Data are not yet available regarding the accumulation of nanoparticles *in vivo*.

Taken together, excretion of nanoparticles (metabolic and elimination processes) remain poorly understood. Some data suggest that not only the chemical composition, but also the size of nanoparticles influences its excretion (Rajagopalan et al., 1996; Qingnuan et al., 2002). Further studies are required to determine half-lives for nanoparticles in several species. As validated kinetic data of nanoparticles become available, more reliable extrapolation to other situations and other species will become possible.

8. Conclusions

Overall, the nanoparticles described in literature have a common characteristic: at least one dimension of their size is less than the arbitrarily chosen 100 nm. The term nanoparticle is perhaps too broad a term to be of use in physiological studies, as it covers a hodgepodge of materials with distinct physical and chemical properties. The shape, charge and size vary among the different particle types. All these aspects can, potentially, influence the kinetic (absorption, distribution, metabolism and excretion) and toxic properties of the particles.

However, it is unclear to what extent the different nanoparticle characteristics contribute to their kinetics. Since common kinetic properties cannot be extracted from the available data, different particle types should be examined carefully. Similar to ordinary chemical substances, the nanoparticle dose at a target site in the body is critical for the observed (adverse) effects. In order to obtain information of the dose of nanoparticles (inside the body), the development of validated (real-time) detection and characterization methods for nanoparticles in biological fluids and tissues is urgently needed. Moreover, quantified nanokinetic data are essential to enable scientists to model effects. If the necessary kinetic data are available for these models, various extrapolations (cross dose, cross species

and route-to-route) might allow quantitative risk assessment (Kuempel et al., 2006).

With time, the efforts in nanokinetic experiments are essential for the safe and reliable application of nanotechnology in consumer products, food, medical products and other applications. Failure to do so might result in the development of negative societal attitudes towards nanotechnology and as a consequence the retardation of the growth of this promising field.

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